The CB_1 allosteric modulator, ORG27569, has the paradoxical effect of increasing the equilibrium binding of CP55,940 (an orthosteric agonist), while at the same time decreasing its efficacy. ORG27569 also acts as an inverse agonist. We have previously used computational methods, synthesis, mutation, and functional studies to identify ORG27569's binding site in the THM3/TMH6/TMH7 region (Shore et al., *ICRS*, **2012**). In this site, ORG27569 promotes an active-like conformation of the CB_1 receptor, explaining ORG27569's ability to increase CP55,940's equilibrium binding. This site explains ORG27569's ability to antagonize CP55,940's efficacy in three complimentary ways: 1) ORG27569 sterically blocks movements of the second extracellular loop that have been linked to receptor activation, 2) ORG27569 sterically blocks a key electrostatic interaction between the third extracellular loop residue K373 and D2.63$^{(176)}$, and 3) ORG27569 packs against TMH6, sterically hindering movements of TMH6 that the Farrens lab have shown to be important to receptor activation. Additionally, we identified a key interaction between ORG27569's piperidine ring nitrogen and K3.28$^{(192)}$ that is required for ORG27569 to act as an inverse agonist.

Using our model of ORG27569 docked in our active state model (in the presence of CP55,940), we designed, synthesized, and functionally characterized 4 analogs of ORG27569 that were designed to test our model and have improved interactions with the receptor. The analogs were functionalized with 3 different goals: 1) to form electrostatic interactions with D6.58$^{(366)}$, 2) to form an aromatic stack with F3.25$^{(189)}$, or 3) to test packing with TMH6-7. Our strategy to form new interactions with D6.58$^{(366)}$ was the most successful, resulting in an analog that is more potent than ORG27569. Interestingly, none of the analogs acted as inverse agonists, suggesting the potential therapeutic promise of CB_1's allosteric site.

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Structural Model of K^+ Channel Activation by the Beta-Gamma Subunits of G-Proteins

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The first known effectors of the βγ subunits of heterotrimeric G-proteins (Gβγ) were the G-protein-gated inwardly rectifying K^+ (GIRK) channels which underlie acetylcholine-induced decrease in heart rate (IK_{ACh}). How Gβγ subunits specifically regulate the conformations of their effector proteins to alter activity is not understood at a molecular level. Although several GIRK crystal structures have been published, attempts to co-crystallize Gβγ have failed precluding knowledge of the reciprocal interactions between the two proteins.

We have employed a computational approach that combines several known methods in protein-protein docking to produce experimentally testable models of the protein complex. The best scoring model of the GIRK1-Gβγ complex predicted a ~1800 Å^2 interaction surface that includes key interactions of the channel's LM and DE loops with Gβ residues that are known to interact with the helical N-terminus of Gα-GDP in the structure of the inactive heterotrimeric G-protein. The channel-Gβγ interactions predicted by the model could be disrupted by mutation of one protein and rescued by additional mutation of reciprocal residues in the other protein. Channel activity was found to be stimulated by Gβγ interactions that enlarged the cleft between the LM and DE loops of the channel and stabilized the LM loop in a ''raised'' position seen in the ''open intracellular gate'' conformation of the GIRK1 crystal structure. GIRK4 displayed differences from the GIRK1 with respect to the pattern of responses to Gβγ mutants but the physiologically relevant heteromeric GIRK1/4 channel behaved similarly to GIRK1. The proposed site of action of Gβγ in the channel's DE-LM cleft is also shared with alcohols and is consistent with a previously described cascade of PIP_2-driven changes in intramolecular interactions of the channel leading to stabilization of the open conformation of its intracellular G-loop gate.

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Construction of a GPR18 Receptor Model using Conformational Memories

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The putative cannabinoid receptor, GPR18, is a member of the Class A subfamily of G-Protein Coupled Receptors (GPCRs). GPR18 binds both lipid-like and small molecule ligands, including NAGly and abnormal-cannabidiol (Abn-CBD) (Kohno et al., BBRC 2006; McHugh et al., Br J Pharmacol 2012). In order to explore the nature of GPR18/ligand interactions, we constructed models of the GPR18 inactive (R) and activated (R*) states, using the μ-Opioid receptor (MOR) crystal structure as template (Manglik et al., Nature 2012). The Monte Carlo/simulated annealing method, Conformational Memories (CM) (Whitnell et. al, J. Comput. Chem. 2007) was used to study the accessible conformations of three GPR18 transmembrane helices (TMHs) with important sequence divergences from the MOR template: TMH3 (P3.36 vs. M3.36 in MOR), TMH4 (L4.54 vs. S4.54 in MOR which participates in a hydrogen bond network that produces a significant bend in TMH4 MOR), and TMH7 (DVILY vs. NPVLY in MOR). We also used CM to calculate the accessible conformations for TMH6 (CFMP vs. CWTP in MOR). This allowed the choice of TMH6 conformers appropriate for the GPR18 R and R* models. All CM calculations used ideal helices as starting points with standard φ (−63°) and ψ (−41.6°) backbone dihedrals. TMH7 calculations also used an ideal helix as starting point, but with a 3(10) helix geometry in the DVILY region. Extracellular and intracellular loop geometries were calculated using Modeller v9.1. Energy minimizations of the resultant R and R* models were performed using the OPLS2005 all atom force field in Macromodel 9.1 (Schrodinger, 2006). The resultant GPR18 R and R* models were used to determine key residues for ligand docking.

2102-Pos Board B121

Aquaporins within a Tetramer Exhibit Different Structural Conformations: An in Silico Study of the Human Aquaporin 5

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Aquaporins are protein channels located across the cell membrane with the role of conducting water or other small sugar alcohol molecules (aquaglyceroporins). The high-resolution X-ray structure of the human aquaporin 5 (HsAQP5) exhibits an important feature: the entire tetramer is crystallized, i.e., the tetramer is not obtained by rotating the monomeric structure around the main axis of the tetramer. Hence, by means of molecular dynamics simulations we conducted a study on the importance of the protein-protein coupling within an aquaporin tetrameric structure and characterized the structural behavior of the human AQP5. We found that different conformations within the tetramer lead to a distribution of monomeric channel structures, which can be characterized as ''open'' and ''closed''. Both the extracellular (where the selectivity filter is located) and the cytoplasmic ends of a channel sample ''closed'' states. In the former region, this can be characterized by a strong narrowing and much lower water permeation rates. In the cytoplasmic end's ''closed'' state water passage is completely blocked by a gating mechanism characterized by the translation of the His67 residue inside the pore. While removing the crystallographic lipid occluding the central pore of the tetramer has no influence on the gating system, the protein-protein coupling might play an important role in regulating its mechanism. Furthermore, our calculated permeation rate of a fully ''open'' channel was found to be in very good agreement with the experimental value.

2103-Pos Board B122

Temperature Controlled Helix-Helix Interactions in Desk Minimal Sensor

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DesK is a temperature sensing transmembrane protein that acts as a molecular switch to regulate membrane fluidity as a function of temperature change. The full function of DesK is modelled by a chimeric construct, denominated minimal sensor (MS), formed by the upper and lower halves of transmembrane helices 1 and 5, respectively. The signalling by MS has been explored experimentally providing data for modelling studies. Resulting from this, the current view is that it forms a dimer, switching its conformation depending on the temperature. To further investigate the molecular details of the switch mechanism, we have developed a new method for exploring the energy landscape of interaction, which allows high throughput screening of transmembrane helix dimers. The results show a clear distinction between helix-helix interactions at high and at low temperatures, providing a molecular basis for the functioning of the minimal sensor. These results form the basis for further experimental exploration, as well as for the rational design of other switching sensors.

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Molecular Basis for the Ion Selectivity of Gap Junction Channels Elucidated by Molecular Dynamics Simulations

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Gap junction channels, formed by 21 types of connexins, are essential for intercellular communications, and their mutations are associated with various diseases. Electrophysiological studies have identified disparate ion selectivity for different connexin channels, but the molecular basis remains unclear.